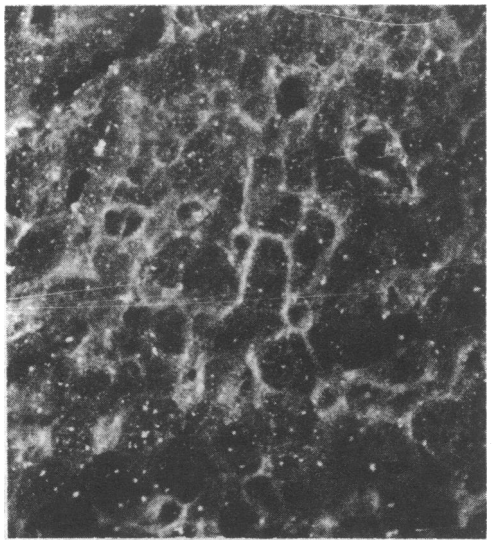

FIG. 2—As fig. 1, showing linear and pericellular deposition of IgG. (× 450.)

polygonal network as described by others (Holborow, 1972) but did not completely reproduce the coarser patchy staining found in the present three livers by direct immunofluorescence.

Viable cell staining on the liver of the third case with active chronic hepatitis and high titre anti-smooth-muscle antibody failed to show surface-bound IgG.

Discussion

The present evidence indicates that IgG may be deposited in the liver of some patients with active chronic hepatitis. All three positive cases possessed features in common, being young women who were untreated at the time of biopsy. All three possessed high titres of IgG anti-smooth-muscle antibody and this may indicate that only the antibody-positive patient has hepatic binding of IgG. Since the serum antibody was of the IgG class in all three cases, possibly anti-smooth-muscle antibody itself is bound within the liver. In view of the findings of Holborow (1972), the present observations might be interpreted in terms of an antigen which crossreacts with smooth muscle. If this is the case, however, the failure of anti-smooth-muscle antibody completely to reproduce the characteristic pattern in normal livers suggests that the antigen must be altered in some way in association with active chronic hepatitis. One of several alternative explanations would be that the IgG is an antibody directed against, and reacted with, some liver-specific antigen unrelated to smooth muscle. Another possibility is that the IgG reflects the deposition of circulating antigen-antibody complexes. Further speculation is premature until it has been shown that the staining pattern is due to the deposition of a specific antibody and until the relevant antigen has been identified.

Work is in progress to determine the precise significance and diagnostic specificity of the finding of IgG, but it is suggested that direct immunofluorescence should be performed on liver biopsies. Humoral mechanisms may prove to be important in the pathogenesis of at least antibody-positive active chronic hepatitis. Recent evidence favouring the involvement of cell-mediated immunity should not be considered in isolation (Miller *et al.*, 1972).

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Changes in Monosaccharide Content of Mucous Glycoproteins in Ulcerative Colitis

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Summary

Colonic mucus appears to consist of two glycoprotein fractions, one of which contains mannose, whereas the other is mannose-free. The mannose-containing fraction is significantly increased in ulcerative colitis.

Introduction

Mucous glycoproteins are secreted throughout the gastrointestinal tract and have protective and lubricatory functions. Chemically they consist of a polypeptide chain to which are attached a large number of oligosaccharide units which surround and protect the protein core from proteolysis. The carbohydrate, therefore, is the most important component in determining the physical, chemical, and biological properties of the molecule. This carbohydrate consists of varying proportions of fucose, mannose, galactose, N-acetylglucosamine, N-acetylgalactosamine, and sialic acid. Endoscopic mucosal biopsy specimens provide a convenient uncontaminated source of mucus, and their carbohydrate composition is almost exclusively contributed by mucus adherent to the epithelial surface or within goblet cells (Brown *et al.*, 1972). Gas liquid chromatography is capable of analysing the monosaccharide content of the mucous glycoproteins from these small mucosal specimens. Studies were also carried out on first-specimen meconium, which is an abundant source of uncontaminated intestinal secretions containing a large proportion of mucous glycoprotein (D. Fraser and J. R. Clamp, unpublished findings), and this was used for the isolation of reasonable quantities of the two major glycoprotein fractions.

Materials and Methods

Mucosal biopsy specimens were collected at colonoscopy or sigmoidoscopy from normal subjects and from patients with ulcerative colitis, chronic diarrhoea, and infective diarrhoea. All specimens were taken from a constant anatomical site—namely, the rectosigmoid junction—using either the Olympus

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CF-LB or the A.C.M. 9000 PL colonoscope or, where these were not available, the Lloyd-Davies sigmoidoscope. The specimens were placed immediately into absolute alcohol and later transferred to a vacuum desiccator and dried to constant weight.

The monosaccharide content of the specimens was determined by gas chromatography (Bhatti *et al.*, 1970). The glycosidically-bound residues were estimated after methanolysis, whereas any free residues were estimated directly after homogenization and aqueous extraction of the specimens. Both free sugars and methyl glycosides were converted to the trimethylsilyl ethers before chromatography. The analyses were carried out on a Hewlett-Packard Gas Chromatograph model 402 using flame ionization detection.

Whole colons were obtained from three patients undergoing colectomy for chronic ulcerative colitis and from two normal subjects within six hours of death. The colons were opened and the mucosal surface was exhaustively washed with 0·15N sodium chloride followed by distilled water. The surface was then scraped and the scrapings, suspended in 0·15N sodium chloride, were subjected to homogenization and ultrasonic disintegration followed by centrifugation at 38,000 *g* for 30 minutes. This extraction procedure was repeated five times and the pooled supernatant solutions were extensively dialysed against distilled water and freeze dried. The dried material was dissolved in 0·15N sodium chloride and subjected to exclusion chromatography on a column of Sephadex G-50. The excluded fraction was dialysed against distilled water and freeze dried. The dried material was dissolved in 0·15N sodium chloride and subjected to further exclusion chromatography on a column of Sepharose 2B using 0·02 M sodium phosphate buffer (pH 7·0). The tubes were pooled according to the hexose profile to give included and excluded fractions.

First specimen meconium was collected from 20 newborn babies. The pooled material was subjected to exhaustive dialysis against distilled water and freeze dried. The dried material was extracted, centrifuged, and chromatographed as described above.

Immunoelectrophoresis was performed on Ionagar No. 2 slides using antisera against whole serum and the immunoglobulin classes.

Results and Discussion

Mucosal biopsy specimens were taken from 52 patients with histologically proved ulcerative colitis. The carbohydrate analyses were compared with those from 46 normal subjects and from 25 patients with chronic noninflammatory diarrhoea. They were also compared with specimens taken from 15 patients with inflammatory diarrhoea of infective origin consisting of eight cases of amoebic dysentery and seven cases of bacillary dysentery.

For comparative purposes the results in the table are expressed as monosaccharide residues relative to six residues of galactose. These results show that there is a highly significant difference ($P < 0.001$) in the mannose content of ulcerative colitis specimens as compared with the other three groups. In one case the finding of a high mannose level in the biopsy specimen preceded the morphological and histological changes of ulcerative colitis.

Mucous glycoproteins from meconium and from normal and colitic colons were separated on Sepharose 2B to give included and excluded fractions, the excluded fraction being greater in the meconium and normal colons. The mannose-containing glycoprotein was present mainly in the included fraction in all three cases. In view of the difference in content of the mannose-containing glycoprotein in ulcerative colitis an attempt was made to establish the source of this material. Serum contains a number of glycoproteins with relatively high contents of mannose, as do secreted immunoglobulins. The Sepharose fractions, however, did not react with antisera against whole serum or against the immunoglobulin classes. In addition if the mannose-containing glycoprotein originated by transudation from serum one would expect the biopsy material to contain serum albumin and free glucose, and neither of these was found in significant amounts in biopsy homogenates. Furthermore, for the glycosidically-bound mannose to be accounted for by serum the entire weight of the biopsy specimen would have to consist of serum glycoproteins and, even if this were possible, the proportions of the other monosaccharides, particularly N-acetylgalactosamine, would be radically altered. Finally, as this difference is not found in infective colitis it is not likely to be due to a nonspecific inflammatory response.

Monosaccharide Content of Colonic Biopsy Specimens. Results are given as Means ± S.D. and expressed relative to 6 Residues of Galactose

	Ulcerative Colitis	Normal	Chronic Diarrhoea	Infective Diarrhoea
No. of patients:	52	46	25	15
Fucose	1·8 ± 0·6	1·9 ± 0·5	1·9 ± 0·5	2·0 ± 0·5
Mannose	5·7 ± 2·5	2·2 ± 0·7	1·9 ± 0·5	2·2 ± 0·5
Galactose	6	6	6	6
N-Acetylglucosamine	6·8 ± 0·8	7·0 ± 0·7	7·3 ± 0·7	7·6 ± 0·8
N-Acetylgalactosamine	4·6 ± 1·0	5·1 ± 1·4	5·0 ± 1·2	4·7 ± 1·1
Sialic acid	5·7 ± 2·0	6·4 ± 2·0	6·3 ± 1·4	5·8 ± 1·2

All this evidence suggests that mucus contains a mannose-rich glycoprotein which normally constitutes only a small proportion of the total mucous glycoproteins produced. The exact origin of this glycoprotein is not known but it may arise from goblet cells or, more likely, from secretory epithelium. Thus the parotid gland, which has very few goblet cells, produces a large number of glycoproteins, most of which have a high mannose content (R. H. Teague and J. R. Clamp, unpublished findings). So far as the function of the mannose-containing glycoprotein is concerned it may be significant (A. Couper, University of Bristol, personal communication) that this component markedly reduces the viscosity of the mannose-free excluded mucous glycoprotein and may therefore be important in regulating the physicochemical properties of the mucus layer. For example, an imbalance of the two components might alter the protective value of this layer in ulcerative colitis and might also be important in other diseases such as cystic fibrosis.

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